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Animal Source Identification Using A *Cryptosporidium* DNA Characterization Technique

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NOTICE

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FOREWORD

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

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This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

Hugh W. McKinnon, Director
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ABSTRACT

This document summarizes the application of a particular molecular method to improve detection and differentiation of species and genotypes of *Cryptosporidium* oocysts found in environmental samples. Of particular interest is the method's potential for determining the source animal types of oocysts in water samples. The molecular method is a nested polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) procedure that characterizes the small sub-unit (SSU) ribosomal RNA gene. The method was previously developed for characterizing oocyst DNA from clinical samples. The current project explores the method's applicability to environmental water samples, which have greater diversity of oocyst species and strains, lower concentrations of oocysts, and different interferents than clinical samples. Results include demonstrating that the method is capable of detection and differentiation of at least 10 species and 22 genotypes of *Cryptosporidium*; method sensitivity demonstrated to a single oocyst with laboratory samples; and detection and differentiation of oocysts from oyster gill washings and hemolymph, storm water, surface water, and raw waste water. The method's capability to determine an oocyst's source animal type was demonstrated by identification in environmental water samples of host-adapted *Cryptosporidium* species and genotypes that were consistent with the source animal types (i.e., humans, farm animals, wildlife, and/or pets) inhabiting the sampled watersheds.

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